PB83-153718

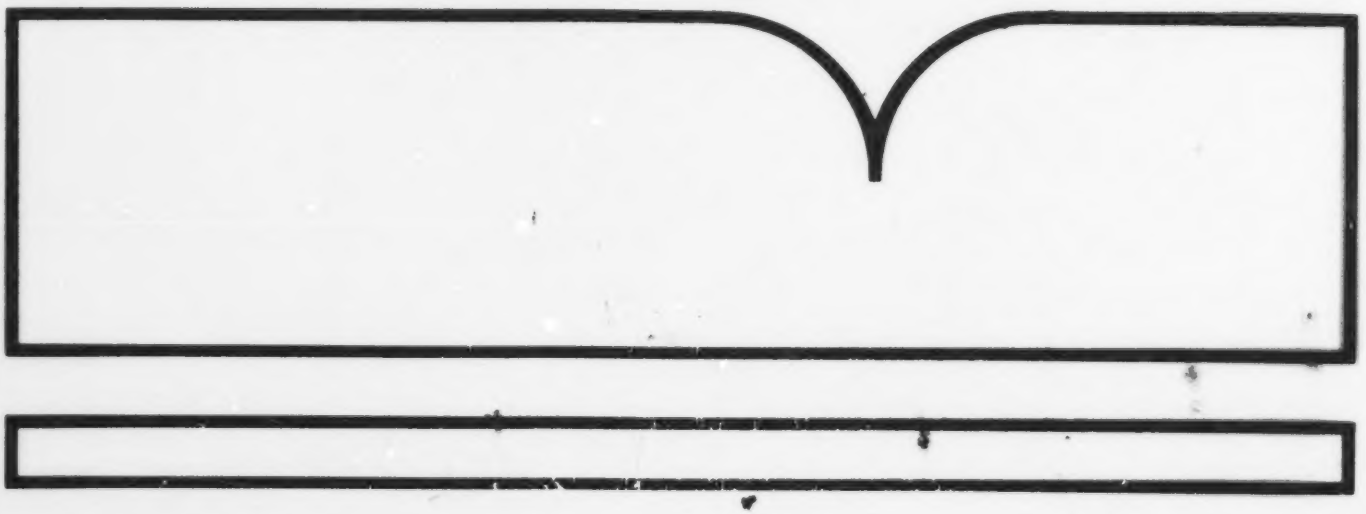
Monitoring Biotransformation and Biodegradation of Xenobiotics in Simulated Aquatic Microenvironmental Systems

Louisina State Univ., Baton Rouge

Prepared for

Environmental Research Lab.
Gulf Breeze, FL

1982



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1. REPORT NO.	2.	3.
EPA-600/J-82-261	Journal Article	PB83-153718

4. TITLE AND SUBTITLE	5. REPORT DATE
MONITORING BIOTRANSFORMATION AND BIODEGRADATION OF XENOBIOTICS IN SIMULATED AQUATIC MICROENVIRONMENTAL SYSTEMS	1982
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
R.J. Portier and S.P. Meyers	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Louisiana State University Baton Rouge, Louisiana 70803	11. CONTRACT/GRANT NO. R804976
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. Environmental Protection Agency Environmental Research Laboratory Office of Research and Development Gulf Breeze, FL 32561	14. SPONSORING AGENCY CODE EPA/600/04

15. SUPPLEMENTARY NOTES

Chapter 42, Developments in Industrial Microbiology 23:459-475

16. ABSTRACT

Microbiological studies coupled with physiochemical analysis of environmentally significant xenobiotics were conducted in continuous flow-through and carbon metabolism microcosms to determine the behavior of these toxic substances in soil and sediment-water systems typical of coastal wetlands. The organophosphate, ^{14}C-methyl parathion, and the chlorinated insecticide, ^{14}C-kepone, were examined for stress indexes based on microbial response and compound turnover. Significant increases in microbial populations, ATP, and specific enzyme system (i.e., phosphatase and dehydrogenase), coinciding with rapid $^{14}CO_2$ expiration and ^{14}C-assimilation by the cellular component, pointed to both rapid biotransformation and biodegradation of methyl parathion. Minimal rates of $^{14}CO_2$ release were noted for kepone, with no significant variation seen in total microbial response or ATP levels. However, ^{14}C-assimilation of kepone was detected. Degradation rates for methyl parathion were comparable to *in situ* rates at experimental plots. Fungal biotransformation is suggested as important in methyl parathion degradation, with a negative response for kepone. Similar activity was discerned for both compounds in axenic flask studies. Statistically valid correlations are evident between microcosm and field data.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	unclassified	16
	20. SECURITY CLASS *(This page)*	22. PRICE
	unclassified	

EPA-600/J-82-261
JOURNAL ARTICLE

Reprinted from Volume 23 of DEVELOPMENTS IN INDUSTRIAL MICROBIOLOGY
A Publication of the Society for Industrial Microbiology - 1982

CHAPTER 42

Monitoring Biotransformation and Biodegradation of Xenobiotics in Simulated Aquatic Microenvironmental Systems

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Microbiological studies coupled with physiochemical analysis of environmentally significant xenobiotics were conducted in continuous flow-through and carbon metabolism microcosms to determine the behavior of these toxic substances in soil and sediment-water systems typical of coastal wetlands. The organophosphate, ^{14}C-methyl parathion, and the chlorinated insecticide, ^{14}C-kepone, were examined for stress indexes based on microbial response and compound turnover. Significant increases in microbial populations, ATP, and specific enzyme systems (i.e., phosphatase and dehydrogenase), coinciding with rapid $^{14}CO_2$ expiration and ^{14}C-assimilation by the cellular component, pointed to both rapid biotransformation and biodegradation of methyl parathion. Minimal rates of $^{14}CO_2$ release were noted for kepone, with no significant variation seen in total microbial response or ATP levels. However, ^{14}C-assimilation of kepone was detected. Degradation rates for methyl parathion were comparable to *in situ* rates at experimental plots. Fungal biotransformation is suggested as important in methyl parathion degradation, with a negative response for kepone. Similar activity was discerned for both compounds in axenic flask studies. Statistically valid correlations are evident between microcosm and field data.

INTRODUCTION

The impact of toxic chemicals on the environment and the need to consider such factors in a variety of land-use and disposal decisions are well recognized. Usable analytical predictive approaches, or protocols, are needed to discern and resolve problems between maintenance of regions of aquatic productivity and chemical discharges from diverse industrial sources as well as sites of accidental spills and waste disposal. A major difficulty in such assessment studies is to obtain reproducible and valid test information for monitoring of critical factors to allow accurate analysis of the particular event. Ideally, the approach should combine field and laboratory investigations, with the latter designed to simulate the "target" environment and introduce relevant biological, chemical, and physico-chemical elements that significantly affect substrate turnover and microbial processes.

The value and application of the microcosm approach in assessing effects of toxic chemicals are well documented (Hague 1980). Microcosms provide a correlated interpretative approach to field studies in aquatic environments, wherein they contribute needed data on fate and transport of short-lived biologically significant compounds. The microcosm system, developed in our laboratory (Portier 1979), can be modified to meet specific requirements, i.e.,

aerobic vs. anaerobic, ^{14}C respiration, rate/fate analyses, etc., and is designed to monitor a variety of physico-chemical factors comparable to these of *in situ* environments. In this and other work, correlations between laboratory and *in situ* studies have demonstrated the reliability of the microcosm as an analytical tool. Data correlations have been developed, along with a statistical and computer program, allowing input of a variety of factors on microcosm response and its predictive value. It should be noted that methodologies developed for terrestrial studies generally are not applicable to fate and transport investigations in aquatic regimes.

This present study presents additional information from our microcosm investigations on development of predictive models of the biotransformation and biodegradation of a variety of xenobiotics in soil and sediment-water systems typical of coastal wetlands. The experimental approach combines field and laboratory (microcosm) methods, with the latter employing a variety of determinants, including microbial indexes, enzymatic activities, respiratory response, and transformation of key substrates such as chitin and cellulose (Portier and Meyers 1981a, b). Ultimately, this composite information will be used in further development of analytical and statistical mathematical models, protocols for analyses of xenobiotics and their influences on critical environmental processes.

Materials and Methods

Sample collection. Samples were taken in the Lac des Allemands environment of the Barataria Bay drainage basin, Louisiana. This watershed region comprises distinctive vegetative zones and areas of contrasting salinity, all affected by a Gulfward movement of water. The brackish and freshwater regions, located adjacent to intensively cultivated agricultural fields, are most directly affected by runoff.

Coring devices, consisting of aluminum cylinders, 60 cm tall by 7.6 cm diameter, were inserted into the sediment to the water level, capped and sealed, and stored on ice for transportation to the laboratory for subsequent microbial analysis. In drier sites, an inclined trench to a depth of 60 cm was dug with an undisturbed wall at one end for aseptic sampling. All samples were processed within a 12-h period. Substrate from the particular sites was used to inoculate individual microcosms and to categorize the major microbial groups present.

Enumeration of microorganisms. For enumeration of total heterotrophic aerobes and anaerobes, sediment samples were appropriately diluted with phosphate buffer, pH 7.2. Using the pour-plate method, replicate 0.1-ml samples were added to standard plate-count agar (Difco), incubated for 3d at 26 C, followed by enumeration of colony-forming units (CFU). For anaerobic studies, proper atmospheric conditions were maintained using a disposable hydrogen/carbon dioxide envelope (Gaspak) in the presence of a cold catalyst in an anaerobic jar. A methylene blue indicator strip was used to insure anaerobiosis. Plates were incubated at 36 C and examined every 24 h.

Four general groups of microorganisms, i.e., bacteria, actinomycetes, filamentous fungi, and yeasts, were enumerated based on colony-forming units (CFU) as described earlier (Portier and Meyers 1981a). For enumeration of bacteria and actinomycetes, replicate 1.0-ml aliquots were inoculated on Jensen's (1930) agar medium. Cycloheximide (Sigma), an antifungal agent, was added at 40 μg/ml to inhibit overgrowth by filamentous fungi. For enumeration of filamentous fungi and yeasts, Martin's (1950) agar medium was used together with streptomycin (Sigma) and chlorotetracycline (Sigma) at 30 μg/ml to retard bacterial growth. All plates were incubated at 30 C and examined after 3 d. Colonies were enumerated under a dissection microscope to distinguish morphologically the four microbial groups.

Enzyme assays. Phosphatase activity was determined using the procedure outlined by Tabatabai and Bremner (1969), and modified by Atlas et al. (1977). Replicate 2-g aliquots of control and pesticide-treated sediments were placed in 150-ml Erlenmeyer flasks with 0.1 ml of toluene, 4 ml of sterile, distilled, deionized water, and 0.5 mg/ml of *p*-nitrophenol phosphate. Flasks were incubated at 30 C for 100 min. With the addition of 1 ml of 0.05 *M* calcium chloride, 4 ml of 0.5 *M* sodium hydroxide, and 20 ml of distilled deionized water, each sample was centrifuged at 8,000 rpm for 10 min and the resulting yellow solution assayed spectrophotometrically (PYE-UNICOM 1700) at 410 nm for units of *p*-nitrophenol produced.

Dehydrogenase determination was based on formation of 2,3,5-triphenyltetrazolium (TPF) from a standard of 2,3,5-triphenyltetrazolium chloride (TTC). Replicate 10-g aliquots of control and pesticide-treated soils were placed in 250-ml Erlenmeyer flasks and brought to 100% water-holding capacity with the addition of a 0.5% solution of 2,3,5-triphenyltetrazolium chloride (Sigma), as described by Bartha et al. (1967). Each flask was flushed with 0_2-free nitrogen, tightly stoppered, and incubated for 6 h (Casida 1977). The TTC is converted by dehydrogenase enzymes to 2,3,5-triphenyltetrazolium formazan, forming a characteristic red color. The latter was extracted with 50 ml of spectroscopic grade methanol (Baker Chemical), centrifuged, and filtered through No. 5 filter paper (Whatman). Absorption was measured at 485 nm and dehydrogenase activity units expressed as units of mg/ml TPF.

Adenosine triphosphate assay. An adenosine triphosphate (ATP) assay, as advanced by Holm-Hansen and Booth (1966) and further presented by Karl (1980) and Stevenson et al. (1979), was used for determination of microbial biomass. In our earlier unpublished investigations, releasing reagents from a variety of commercial manufacturers, designed for specific release and quantification of microbial and somatic ATP, were used. These reagents allow selective permeable release of nucleotides without cell lysis. Thus, nucleotides are released without being broken or changed by the enzymes of the cells, and ATP measurement can be achieved with minimal interfering effects as noted by Stevenson et al. (1979). Excellent reproducible assays were achieved with a short preparation time. Use of selective applications of these reagents (somatic and microbial) provides good differential quantifications of ATP. Only 200 μl

of sample was needed for easy assay. The responsiveness of this ATP assay approach to toxic substances input is being reported elsewhere.

Microcosm design. A regulated, continuous flow-through microcosm of model ecosystem (Portier and Meyer 1981a) has been developed and evaluated in our laboratory to discern substrate rate effects and stress indexes based on microbial and enzymatic response and compound turnover. Evaluations have included time/activity analysis and data validity determinations with suitable replication. The static microcosm is designed for $^{14}CO_2$ studies and "tagged" substrate turnover (Fig. 1) and allows study of the effects of xenobiotic compound addition on respiration rate and biomass accumulation of labeled ^{14}C-compounds.

Basically, features of the microcosm permit variability in flow rate, temperature, and pH/Eh; permit maintenance of a variety of soil/water relationships analogous to natural conditions; allow for the input and outflow of a variety of test solutions. The entire system is autoclavable, thus allowing axenic tests, and is capable of maintaining selected temperature, flow rate, and pH for 5 wk or longer.

Microcosm investigations. For the controlled studies, sediment from the Lac des Allemands site was placed into a series of continous flow-through microcosms. Approximately 50 g (wet wt) of sediment were introduced into each reaction chamber with appropriate amounts of filter-sterilized (0.45 μm cellulose acetate membrane) site water to a final volume of 1 liter. One battery of four microcosms served as controls while solutions of the test compound, organophosphate or organochlorine, was introduced into the second battery of microcosms following equilibration. The test solution, added at a flow rate of 0.105 ml/min, consisted of filter-sterilized site water followed subsequently by site water amended with organophosphate or organochlorine for test microcosms. Temperature was maintained at 29 C with pH at 7.2 (site pH). Temperature and pH were effectively controlled using a Versa Therm Model No. 2158 and Horizon Model No. 5997, respectively. The organophosphate used was methyl parathion (Chem Service) at a stock solution of 5 ppm, while the organochlorine was kepone (Applied Science Labs, Inc.) at a stock solution of 0.5 ppm. These compounds were chosen for their dissimilarities in chemical structure and recalcitrance and for the relevancy of information that could be obtained in terms of realistic environmental situations.

For microcosm studies of carbon metabolism with both xenobiotics, sediment from the field site was dispensed into replicate series of four static microcosms with 500 ml of filter-sterilized site water for each compound. In studies with methyl parathion, a uniformly labeled ^{14}C-methyl parathion solution (Pathfinder Laboratories) was used with a nonlabeled solution (Chem Service) to give a final solution of 5 ppm (1.0 μCi/500 ml). For similar studies with kepone, a ^{14}C-kepone solution (Pathfinder Laboratories) was used with a nonlabeled solution (Applied Science Labs, Inc.) to give a final solution of 0.5 ppm (1.0 μCi/500 ml).

A moderate airflow into each microcosm allowed for entrapment of $^{14}CO_2$

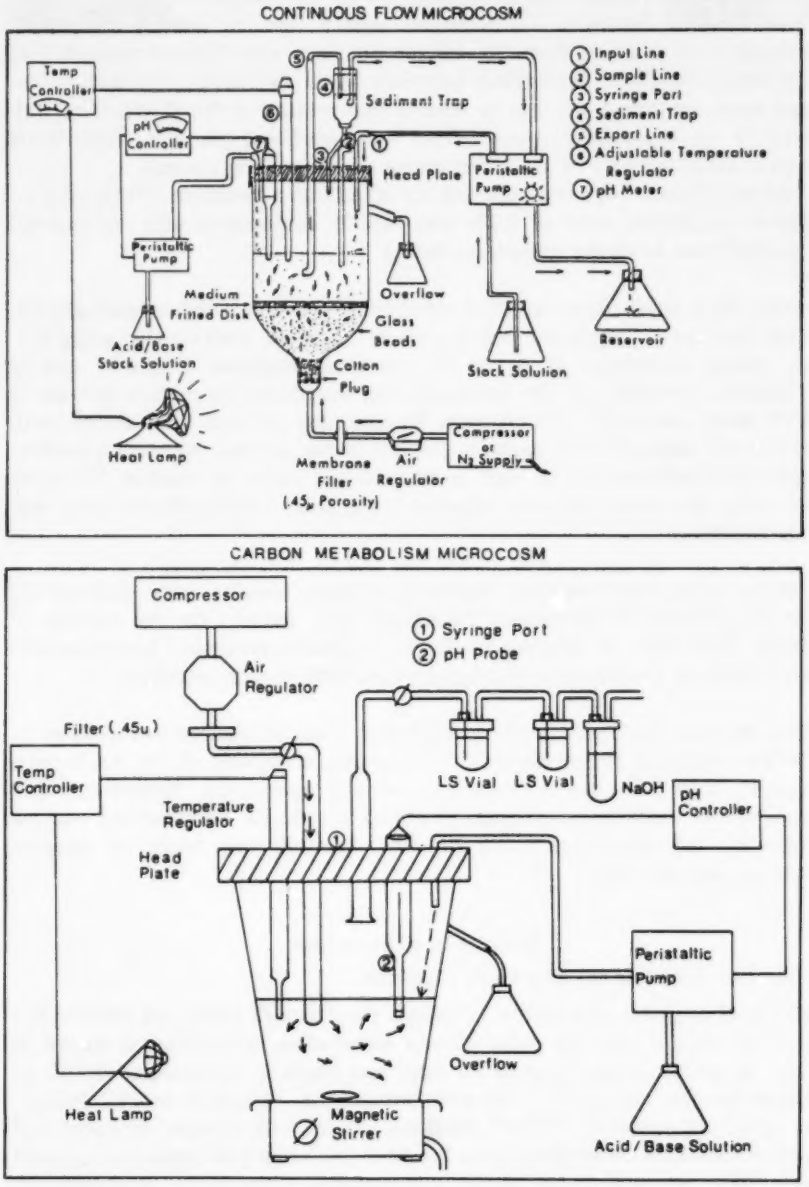

FIG. 1. (a) Diagram of continuous flow microcosm; (b) carbon metabolism microcosm.

by liquid scintillation vials containing 15 ml of complete oxidizer cocktail (New England Nuclear). To determine uptake rates of labeled compound, replicate 1-ml aliquots from 10^5 sample dilutions were filtered through 0.45 μm membrane filters. Each filter was then rinsed with sterile, distilled, deionized water (buffered, pH 7.2) to remove that portion of the chemical not utilized by the microbial biomass. Filters were dried and placed in scintillation vials containing 15 ml of phase-combining scintillant (Amersham).

All scintillation vials were counted for 10 min on a Beckman 250 liquid scintillator. A present error of 0.2% was used in conjunction with the external standard ratio mode for quench correction.

Axenic flask study. Representative members of the four morphological groups, from both methyl parathion and kepone microcosms, were isolated using axenic culture techniques to screen for possible compound sensitivity and/or utilization. Sensitivity to the xenobiotic was determined by relative changes in ATP levels compared with controls. Uptake rates of each radiolabeled compound was determined by methods outlined in the previous microcosm section. Total biotransformation of each compound was based on residual ^{14}C levels following incubation and was adjusted for possible abiotic effects using control chambers.

Gas-chromatography analysis. Residual sediment samples were analyzed via gas-chromatography techniques (Gambrell, pers. comm.) for the amount of parent compound as affected by biotic or abiotic processes. Identifications were based on comparisons with known standards of each xenobiotic.

Data analyses. Statistical methods used have included multivarient analyses to test the validity and reproducibility of the design features of the microcosm, together with SAS and Fortran programming approaches. Evaluations and tests of the variables considered, including population composition, enzyme response, and tagged compound turnover, have all been based on repeated runs of replicate sets.

Results and Discussion

Microbial Response to Xenobiotic Addition

Microbial response to addition of methyl parathion (5 ppm) and kepone (0.5 ppm) in control and test static carbon metabolism microcosms is shown in Figs. 2a and 2b. Data are given for fungi and bacteria, expressed as the log of colony-forming units (CFU), for both compounds. Statistical analysis indicated significant variations in both microbial groups with a single application of methyl parathion. After incubation for 10 d, microbial concentrations returned to initial levels, with no significant changes noted subsequently. No significant total treatment variation was seen in the kepone microcosms of static carbon metabolism with either bacteria or fungi, although bacterial CFU were higher in the control microcosm at day 3 and fungal CFU were higher in control microcosms at day 7. Nevertheless, in general, control microcosm chambers

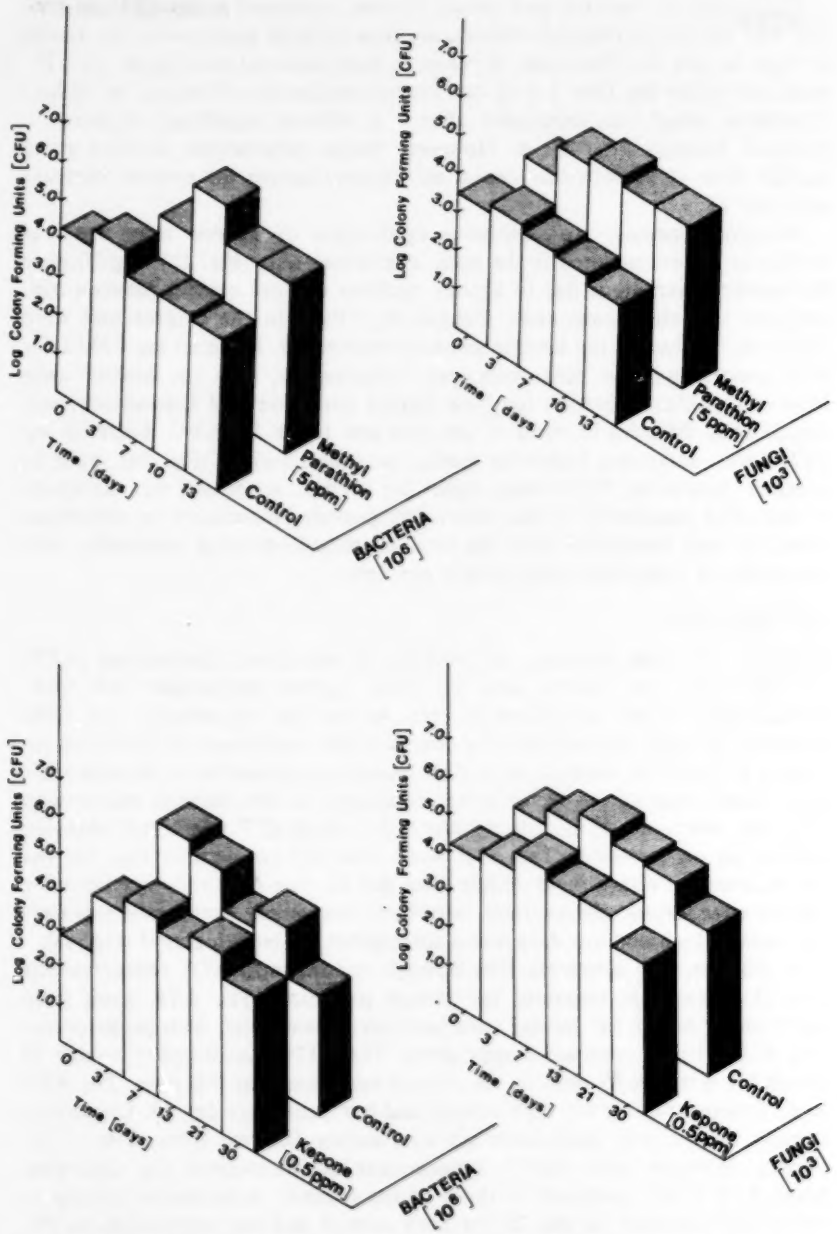

FIG. 2. (a) Microbial response to addition of methyl parathion (5 ppm), static microcosm; (b) microbial response to addition of kepone (0.5 ppm), static microcosm.

had higher CFU than did experimental units.

Fluctuations in bacterial and fungal biomass, expressed as log CFU, in control and xenobiotic-amended continuous flow-through microcosms are shown in Figs. 3a and 3b. Significant increases in both bacterial and fungal log CFU occurred within the first 3 d of continuous application of methyl parathion. Xenobiotic input was terminated after 7 d with no significant variation in bacterial biomass after 10 d. However, fungal populations declined more rapidly after 10 d, with the control microcosms having the greatest decrease after day 17.

Microbial response to continuous application of kepone (Fig. 3b) was similar to responses noted in the static microcosm tests (Fig. 2b). Significance by treatment variations due to kepone addition was not evident between control and test microcosm units. Fungal log CFU's in the control unit were higher at 7 d than in the kepone-amended microcosm. Bacterial log CFU's for both control and test microcosms were comparable, with the control units somewhat higher. Controls for both methyl parathion and kepone were not significantly different in terms of bacterial and fungal log CFU. Bacterial log CFU's were somewhat higher for methyl parathion initially (Fig. 3a), while in contrast, fungal log CFU's were higher for kepone. It appears that variations in microbial populations in the microcosm provide information on xenobiotic selectivity and interaction with the total microenvironmental community and indication of compound recalcitrance and fate.

ATP Responses

Estimates of viable biomass, as provided by adenosine triphosphate (APT) concentrations, for control and test static carbon metabolism and flow-through microcosms are shown in Figs. 4a and 4b, respectively. Log CFU estimates for static microcosms (Fig. 4a) provided indications of significant increases in microbial biomass for methyl parathion, in contrast to kepone addition. These observations were further confirmed in flow-through microcosms (Fig. 4b), wherein a significant increase in microbial ATP was noted following application of parathion. The ATP levels remained consistently high for the test microcosm, with a rapid decline after day 13. The ATP concentrations for kepone were statistically invariant, with both control and test microcosms having variable high and low data points throughout the growth period (Fig. 4a).

In Fig. 4b, for continous flow-through microcosms, ATP concentrations showed comparable responses for methyl parathion. The ATP levels were significantly higher for methyl parathion microcosm units, with peaks occurring within 3 d of continuous application. The ATP levels dropped by day 10 except for a transitory peak in the control microcosms at this time. The ATP levels dropped rapidly for both control and test units after day 10. Concentrations in kepone units again were not significantly different from those of the control. However, after day 3, kepone-amended microcosms had somewhat lower ATP levels compared to those of the control. A moderate increase in ATP concentrations on day 21 for both control and test microcosms is difficult to explain. This may be due to a containerization effect of the microcosms or to subsequent degradation of the lignocellulosic component of

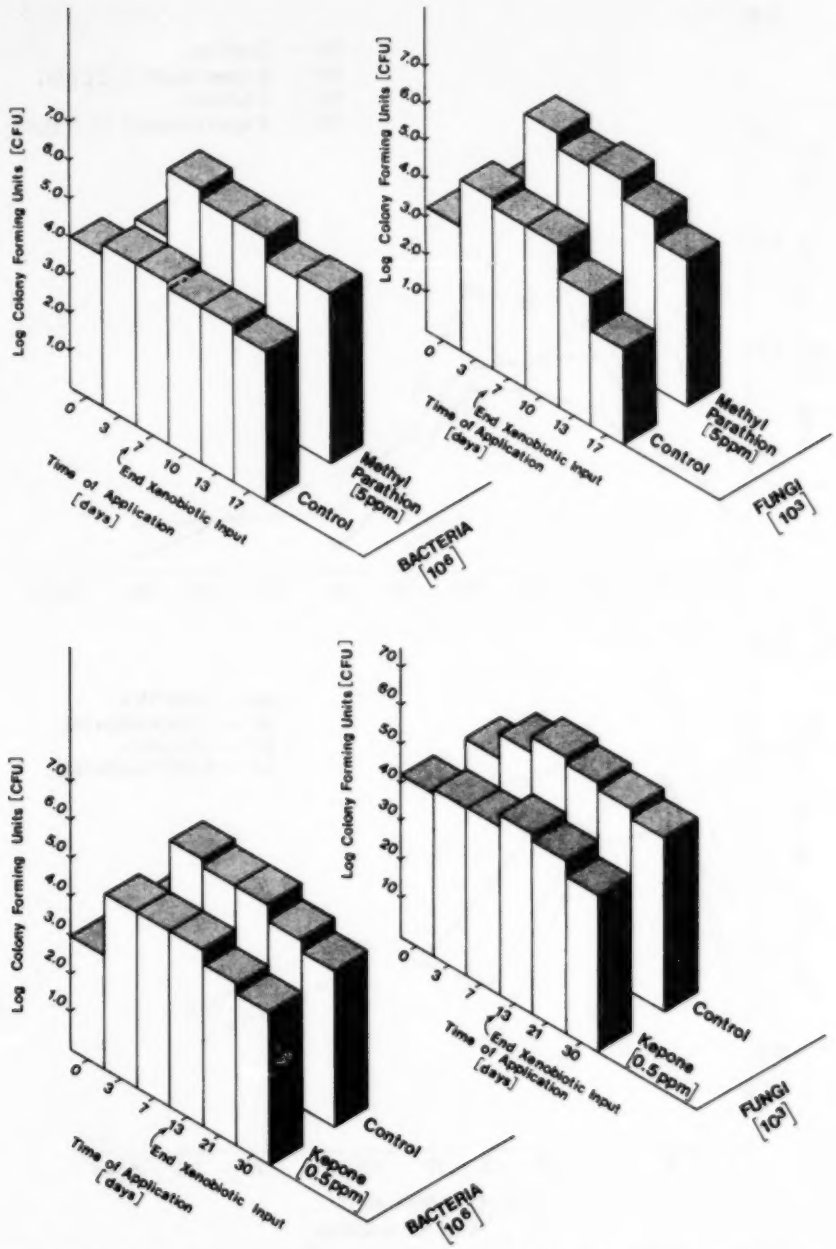

FIG. 3. (a) Microbial response to addition to methyl parathion (5 ppm), flow through microcosm; (b) microbial response to addition of kepone (0.5 ppm), flow through microcosm.

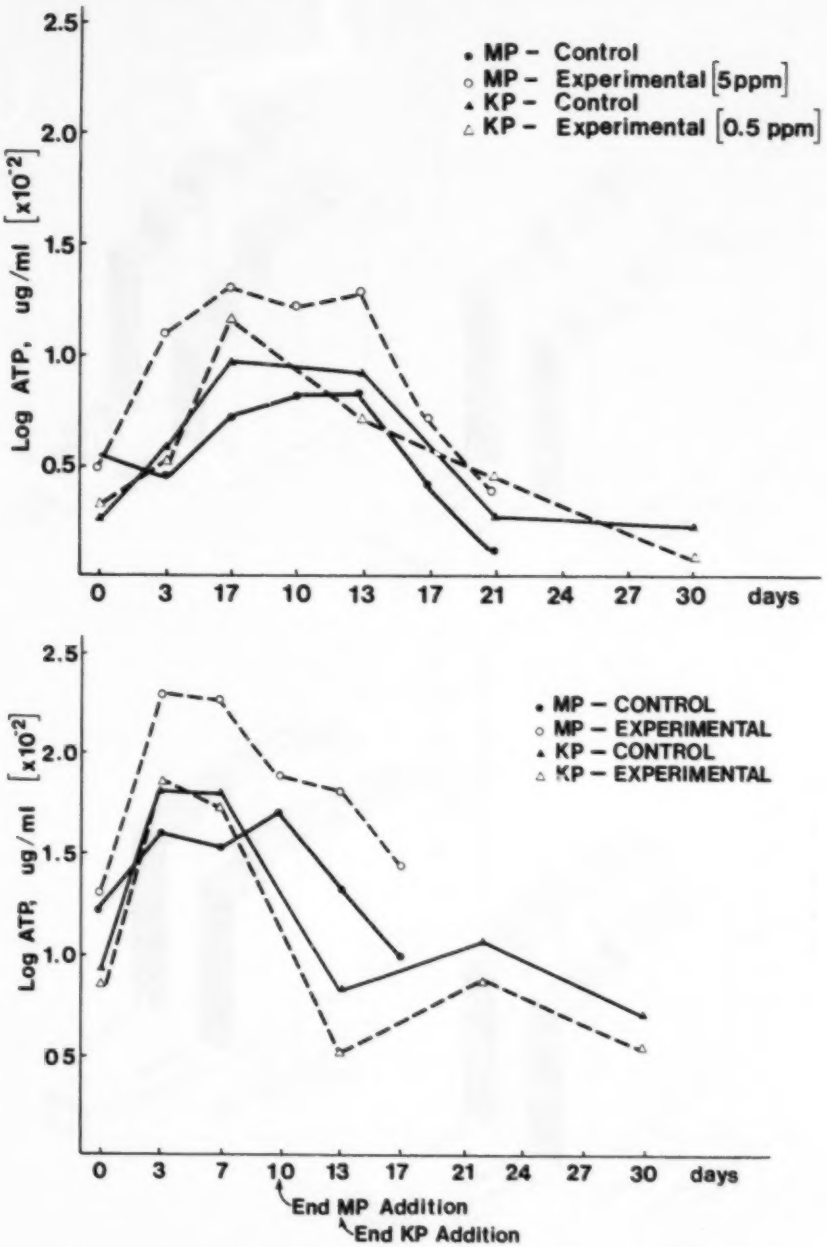

FIG. 4. (a) ATP response to addition of methyl parathion and kepone, static microcosm; (b) ATP response to addition of methyl parathion and kepone, flow-through microcosm.

the sediment. Probably, kepone itself was not solely responsible for this ATP increase although a slight increase in $^{14}CO_2$ release in static microcosms was noted during this time.

Enzyme Responses

A notable decrease in phosphatase activity was seen between kepone-amended and control static microcosm units (Fig. 5a). Phosphatase levels, initially

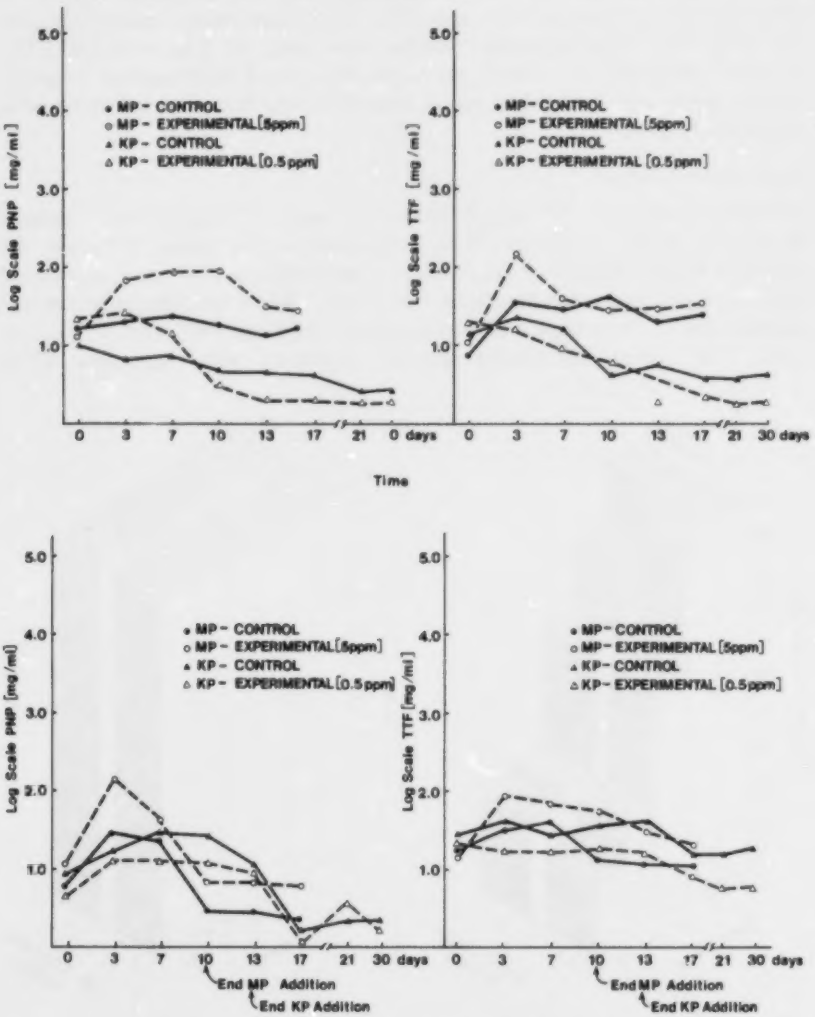
Log Scale PNP [mg/ml]
Log Scale TTF [mg/ml]
MP - CONTROL
MP - EXPERIMENTAL [5ppm]
KP - CONTROL
KP - EXPERIMENTAL [0.5 ppm]
0 3 7 10 13 17 21 0 days
0 3 7 10 13 17 21 30 days
Time
End MP Addition
End KP Addition

FIG. 5. (a) Enzyme (phosphatase and dehydrogenase) response to methyl parathion and kepone, static microcosm; (b) Enzyme (phosphatase and degydrogenase) response to methyl parathion and kepone, flow-through microcosm.

higher than that of controls for kepone, steadily dropped after day 3. In contrast, phosphatase levels for methyl parathion were again significantly higher than controls after day 3. Levels of dehydrogenase for methyl parathion were higher at day 3 but subsequently dropped to, or below, control levels. Hence, no statistical significance for methyl parathion by treatment variation was discerned. Dehydrogenase levels for kepone, although somewhat lower than controls, were not statistically significant.

Significant increases in both phosphatase and dehydrogenase activity in flow-through microcosms were noted for continuous methyl parathion addition (Fig. 5b). These increases coincided with those for total microbial CFU, as noted previously for methyl parathion (Fig. 3b). Dehydrogenase levels for kepone again were somewhat below control values, but were not statistically significantly lower.

Radiotracer Analysis

Residual levels of ^{14}C-methyl parathion and ^{14}C-kepone for replicate microcosm series, based on $^{14}CO_2$ expiration of the parent molecule, are shown in Fig. 6. After 13 d under microcosm conditions outlined previously, a significant loss of methyl parathion was evident. Rapid biotransformation and degradation of the parent molecule occurred within the first week of application. This initial degradation period correlated with increasing CFU,

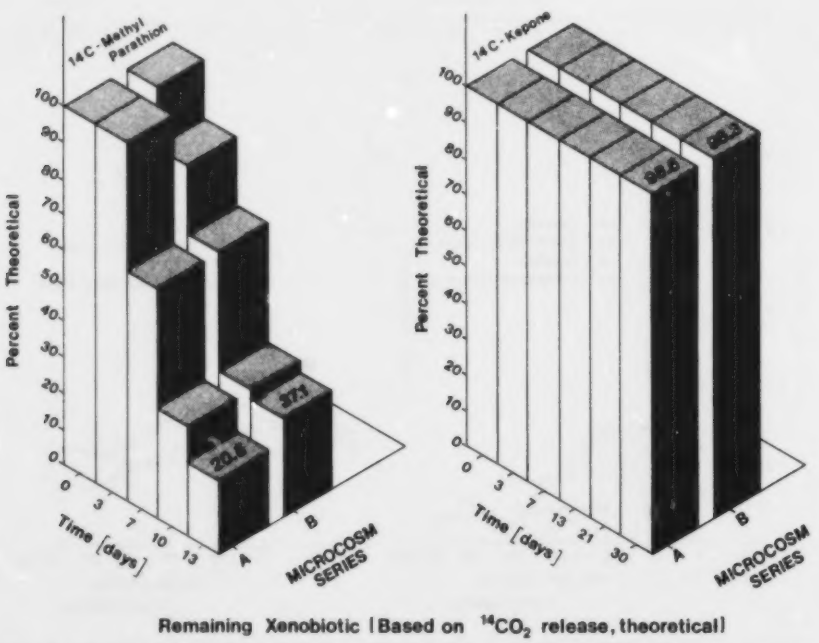

FIG. 6. Residual levels of ^{14}C-methyl parathion and ^{14}C-kepone, based on $^{14}CO_2$ expiration, static microcosms.

phosphatase, dehydrogenase, and ATP levels, as mentioned previously. Figure 7 shows maximal ^{14}C-assimilation of both parent molecules, or their major metabolites, within the CFU, occurring by day 7 for methyl parathion. A sharp decrease is noted in ^{14}C-assimilation by day 13, along with similar decreases for $^{14}CO_2$ levels.

Extremely low levels of $^{14}CO_2$ were detected for kepone during the 30-d incubation time period (Fig. 6). Over 98% of the original kepone, or possibly major metabolites, remained after this period. However, ^{14}C-assimilation of kepone gradually increased during this period, raising the possibility of its being actively assimilated by the microbial component, however, with minimal utilization.

Axenic Flask Studies

Growth responses of representative microcosm isolates are shown in Table 1. In all instances with methyl parathion, a moderate to high response (>30% change) was noted in overall ATP levels. This correlated with rapid assimilation of the parent molecule in mixed enrichment media as designated and a notable total biotransformation. A low response was evident for isolates No. 1, 2, and 3 for methyl parathion in minimal salts media, whereas a negative response occurred for the bacterial isolate No. 4 along with a moderate response in the chitin medium. Although not shown in Table 1, the most appreciable loss of methyl parathion occurred with the fungal Isolate No. 1, 38% after 7 d in Martin's media. However, with methyl parathion as the sole carbon source, only low responses were noted. These results are comparable to those of Rao and Sethunathan (1974) whereby, using enrichment culture tech-

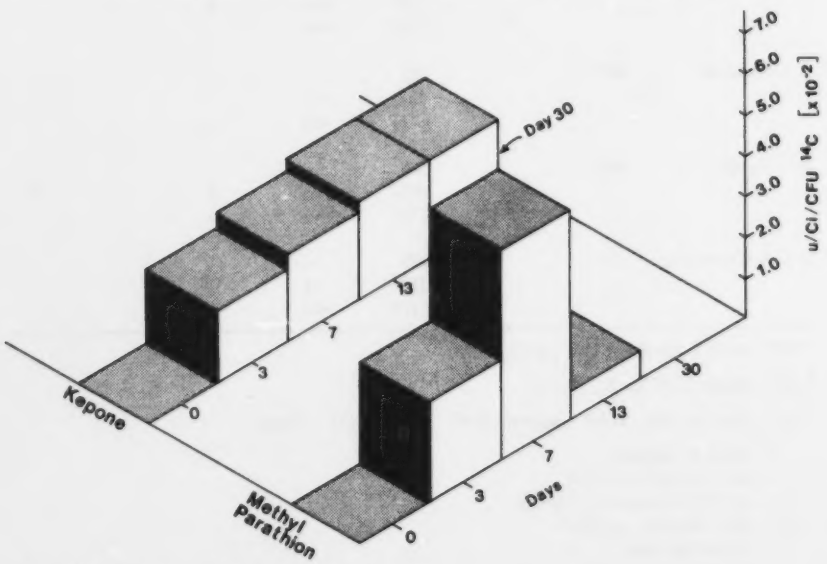

FIG. 7. ^{14}C assimilation within CFU.

TABLE 1. *Microcosm-isolated species level response to tagged xenobiotic addition*

Isolate No.	Microcosm Source	14C-KP[a] + Enrichment	^{14}C-KP[b] + Enrichment	ATP Response	^{14}C Assimilation	Total ^{14}C Biotransformation
1 - Fungal	MP	MS[c]		+	+	+
		MAR[c]		+++	+++	+++
		CHT[c]		+++	+++	+++
2 - Yeast	MP	MS		+	+	+
		MAR		+++	+++	+++
		CHT		+++	++	++
3 - Actinomycete	MP	MS		+	+	+
		JEN[c]		++	++	++
		CHT		+++	+++	+++
4 - Bacterial	MP	MS		-	-	-
		JEN		+++	+++	+++
		CHT		++	++	++
5 - Fungal	KP		MS	-	-	-
			MAR	+++	+	-
			CHT	+++	+	-
6 - Yeast	KP		MS	-	+	-
			MAR	+++	+	-
			CHT	+++	+	-
7 - Actinomycete	KP		MS	-	-	-
			JEN	+++	+	-
			CHT	+++	+	-
8 - Bacterial	KP		MS	-	-	-
			JEN	+++	+	-
			CHT	+++	+	-
1 - Fungal	MP		MS	-	-	-
			MAR	+++	+	-
			CHT	+++	-	-
3 - Actinomycete	MP		MS	-	-	-
			JEN	++	+	+
			CHT	+++	+	+

[a] MP - methyl parathion (500 μg/L).

[b] KP - kepone (100 μg/L).

[c] MS - minimal salts; MAR - Martin; JEN - Jensen; CHT - Chitin.

\- negative response
\+ low response (<10%)
++ moderate response (<30%)
+++ high response (>30%)
incubation time - 7 d
incubation temperature - 30 C

niques, the fungus *Penicillium waksmani* tolerated high levels of parathion and actively converted parathion to aminoparathion. This would indicate, similar to our observations, the important role that fungal species may play in sequential biotransformation of a xenobiotic. Munnecke and Hsieh (1976) alluded to this by using specifically adapted, mixed microbial cultures for the detoxification of parathion.

Orndorff and Colwell (1980) noted the distribution and characterization of kepone-resistant bacteria in the aquatic environment. All representative microcosm isolates grown in the presence of kepone showed minimal sensitivity to the compound at a concentration of 100 μg/L. Relatively low levels of ^{14}C were assimilated by each isolate during the 7-d incubation period. Total ^{14}C biotransformation was not noted for any of the kepone microcosm isolates. However, the actinomycete isolate from the methyl parathion microcosm induced a noticeable total ^{14}C biotransformation, suggesting possible xenobiotic biotransformation by the actinomycete organism. Bourquin et al. (1977) mentioned the possible interference of methyl parathion degradation by kepone. While species-specific responses to xenobiotic input are noted in axenic culture, extrapolation of the data to provide information on the metabolic integrity of an aquatic system may provide an incomplete picture.

Gas Chromatography Analysis

Residual sediment samples, analyzed for methyl parathion via GC techniques, showed close correlations between laboratory and *in situ* rates at experimental plots. The latter ranged from 3.7 to 5.2 d for pesticide half-life. Microcosm half-life values for methyl parathion were 3.3 d (mean treatment determination). Field applications of kepone were not conducted. However, GC analysis of microcosm sediments showed appreciable accumulation levels of kepone in flow-through systems, and neglible loss in static microcosm systems (92.4% remaining).

Conclusions

Application of microcosm approaches to the aquatic environment requires a calibrated, reproducible simulation of the microenvironment in question as well as a demonstration of the effectiveness of the system in providing data on biotransformation and fate of toxicants. The information reported here and elsewhere, using a combination of microbial, enzymatic, and radiotracer approaches, suggests the validity of benchtop microcosms as an accurate analytical "tool." Varying rates of utilization and biotransformation of two dissimilar chemical toxicants, i.e., methyl parathion and kepone, can be discerned within the microcosm. The rapid disappearance of methyl parathion coincides closely with our field results. The ATP and other enzyme tests provide extremely sensitive indications of initial biotransformation of the parent compound, and can be used as early indexes for fate analysis. Axenic flask studies further confirmed these findings.

Our data indicate it is possible to use these approaches to monitor effect

and fate of short-lived toxicants. It is equally important in ascertaining the application of a microcosm approach to establish whether recalcitrant compounds, such as kepone, will behave similarly in the laboratory microenvironment. A rapid biodegradation rate for such a recalcitrant would indicate a containerization effect attributable to either incorrect parameter assessment or to microcosm design itself. As our studies have shown, kepone was very recalcitrant, with minimal $^{14}CO_2$ expiration. The ATP and specific enzyme systems, i.e., phosphatase and dehydrogenase, supported this finding together with lack of significant variations in both bacterial and fungal components of the microbial community. However, levels of ^{14}C assimilation of kepone increase during incubation. Furthermore, kepone accumulated to fairly high levels in flow-through systems, with GC analysis or residual sediments showing low degradations rates. Again, axenic flask studies of representative microbial groups confirmed these results.

Maintenance of the metabolic integrity of an ecosystem (Bourquin 1977), and in particular its microenvironment, is an important consideration along with relative biodegradability of the xenobiotic tested. Microcosms provide a means for replacement of possible sensitive microcoorganisms by tolerant species, as well as monitoring sequential biodegradation processes among important indigenous microbial populations. Fungal and actinomycete groups appear to be particularly significant in this regard. Further studies of toxicant-induced adaptation responses and species replacement in continuous flow-through and static microcosms of carbon metabolism are currently under investigation. The approaches and experimental data given here should permit further development of a quantitative "baseline" of major microbial groups together with relevant enzyme activities for evaluation of ecosystems within larger ecologically important estuaries.

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